REMARKS

1. Based on the Office Communication, the applicants have further amended the claim 1 to clarify the difference between this application and Rieser's prior art by adding the limitation of removing the additional cells by changing medium.

2. As the Office Action indicated, Rieser et al. teach a bone substitute plate (7) with surface pores (surface roughness). However, The US patent 5634879 clearly stated that "Thus, the prosthesis surface roughness makes difficult or almost impossible a floating of cells which have been deposited on or close to the surface." (column 10, lines 23-25) The US patent further stressed that "It is believed that this roughened surface exposes a greater surface anchoring area to cells for attachment." (column 6, lines 2-4)

   The US patent 5746789 also stated "Depending on parameters, such as disk size, spacing between disks, rotational speed, disk surface roughness, downstream pressure, ambient air conditions, etc., either laminar or (more likely) turbulent boundary layers of air will be established on each side of each of the rotating disks in the set. These parameters will establish the pressure drop across the rotating filter device and should be chosen so that the boundary air layers between any 2 disks overlap or at least touch." (column 8, lines 37-46) The US patent 6372494 mentioned that "the maximum particle size that can be extruded through such needles will be a complex function of at least the following: particle maximum dimension, particle aspect ratio (length:width), particle rigidity, surface roughness of particles and related factors affecting particle:particle adhesion, the viscoelastic properties of the suspending fluid, and the rate of flow through the needle." (column 26, line 42-49)

Three scientific articles attached also demonstrated that the surface roughness would effect the cells attachments. Therefore, one would be very difficult, if not impossible, to recover the cells from the substitute plate (7) of Rieser et al.

3. Rieser et al suggested that "it is not necessary to isolate specific cell types from donor tissue." Furthermore, Rieser et al discredited the filter material in US Pat. No 5326357. Therefore, one of ordinary skill in the art would be very possible not to use the bone substitute plate (7) of Riese et al as a filter. Moreover, how would the one of ordinary skill in the art think the bone substitute for implantation to be used a filter?
4. Even thought the cell space taught by Rieser et al. functions as a filter, the result of recovering efficiency is still far behind from this application because that the surface roughness of bone substitute exposes a greater surface anchoring area to cells for attachment. Therefore, the difference between this application and the prior arts cited would not be obvious.

Accordingly, this application should be placed in condition of allowance. An early Notice to this effect is respectfully expected.

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Attachement

1. Authors: Andrews, Kirstie D. Hunt, John A. Black, Richard A.Title: Effects of sterilisation method on surface topography and in-vitro cell behaviour of electrostatically spun scaffolds.

Source: Biomaterials. 28(6):1014-26, 2007 Feb.

Abstract

Electrostatic spinning is a potentially significant technique for scaffold production within the field of tissue engineering; however, the effect of sterilisation upon these structures is not known. This research investigated the extent of any topographical alteration to electrostatically spun scaffolds post-production through sterilisation, and examined any subsequent effect on contacting cells. Scaffolds made from Tecoflex SG-80A polyurethane were sterilised using ethylene oxide and UV-ozone. Scaffold topography was characterized in terms of inter-fibre separation (ifs), fibre diameter (f.dia) and surface roughness. Cell culture was performed over 7 days with both mouse L929 and human embryonic lung fibroblasts, the results of which were assessed using SEM, image analysis and confocal microscopy. Sterilisation by UV-ozone and ethylene oxide decreased ifs and increased f.dia; surface roughness was decreased by UV-ozone but increased by ethylene oxide. Possible mechanisms to explain these observations are discussed, namely photo-oxidative degradation in the case of UV-ozone and process-induced changes in surface roughness. UV-ozone sterilised scaffolds showed greater cell coverage than those treated with ethylene oxide, but lower coverage than all the controls. Changes in cell attachment and morphology were thought to be due to the changes in topography brought about by the sterilisation process. We conclude that surface modification by sterilisation could prove to be a useful tool at the final stage of scaffold production to enhance cell contact, phenotype or function.

2. Authors: Mitchell, S A. Poulsson, A H C. Davidson, M R. Emmison, N. Shard, A G. Bradley, R H.

Titl: Cellular attachment and spatial control of cells using micro-patterned ultra-violet/ozone treatment in serum enriched media.

Source: Biomaterials. 25(18):4079-86, 2004 Aug.

Abstract Ultra-violet Ozone (UVO) modified polystyrene (PS) surfaces were analyzed by X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), contact angle (CA), optical microscopy (OM) and cell culture experiments. UV/Ozone treatment up to 900 s was used to increase the surface oxygen concentration of PS surfaces from 0% to approximately 35% (unwashed) and 0% to approximately 27% (washed). The observed differences in oxygen concentration, between washed and unwashed surfaces, have been previously attributed to the removal of low molecular weight debris produced in this treatment process. Surface roughness (Rq) is known to affect cellular attachment and proliferation. AFM studies of the UV/Ozone treated PS surfaces show the surface roughness is an order of magnitude less than that expected to cause an effect. UV/Ozone treatment of PS showed a marked change in CA which decreased to approximately 60 degrees after 900 s treatment. The increased attachment and proliferation of Chinese hamster ovarian (CHO) and mouse embryo 3T3-L1 (3T3) cells on the treated surfaces compared to untreated PS were found to correlate strongly with the increase in surface oxygen concentration. Surface chemical oxidation patterns on the PS were produced using a simple masking technique and a short UV/Ozone treatment time, typically 20-45 s. The chemical patterns on PS were visualized by water condensation and the spatially selective attachment of CHO and 3T3-L1 cells cultured with 10% (v/v) serum. This paper describes an easily reproducible, one step technique to produce a well-defined, chemically heterogeneous surface with a cellular resolution using UV/Ozone modification. By using a variety of cell types, that require different media conditions, we have been able to expand the potential applications of this procedure.

3. Authors:Okkerse, W J. Ottengraf, S P. Osinga-Kuipers, B.

Title: Biofilm thickness variability investigated with a laser triangulation sensor.

Source: Biotechnology & Bioengineering. 70(6):619-29, 2000 Dec 20.

Abstract Measurement of the surface roughness and thickness of biological films is laborious and usually destructive, thus hampering research in this area. We developed a laser triangulation sensor (LTS) set-up for the fast and nondestructive measurement of these biofilm parameters during growth. Using LTS measurements, the morphological development of a dichloromethane-(DCM) degrading biofilm

cultured on a wetted-wall column was studied. The measurements show that the biofilm develops faster at the entrance of the reactor. The biofilm consisted of a base film in which microbial colonies were embedded. The biofilm-surface area gradually increased by 23% compared to the bare surface due to the formation of a large number of these colonies. The number and shape of these colonies were followed in time. Using LTS measurements, biofilms distinctly different in surface roughness could be distinguished in a laboratory trickling filter removing DCM from a waste gas. The consequences of the observed surface characteristics for the reaction-diffusion process in the biofilm and for the falling film hydrodynamics are discussed.